Vulnerability curves by centrifugation: is there an necessarily invalid?

JOHN S. SPERRY[1], MAIRGARETH A. CHRISTMAN[2], JOSE M. TORRES-RUIZ[3], HARUHIKO TANEDA[4] & DUNCAN D. SMITH[1]

[1]Biology Department, University of Utah, 257S 1400E, Salt Lake City, UT 84112, USA, [2]Institute for Ecohydrology Research, 1111 Kennedy Place Suite 4, Davis, CA 95616, USA, [3]Instituto de Recursos Naturales y Agrobiología (IRNASE-CSIC), Avenida de Reina Mercedes n.°10, 41012 Seville, Spain and [4]Department of Biological Sciences, Graduate School of Science, University of Tokyo, 7-3-1, Hong, Bunkyo, Tokyo 113-0033, Japan

ABSTRACT

Vulnerability curves using the 'Cavitron' centrifuge rotor yield anomalous results when vessels extend from the end of the stem segment to the centre ('open-to-centre' vessels). Curves showing a decline in conductivity at modest xylem pressures ('r' shaped) have been attributed to this artefact. We determined whether the original centrifugal method with its different rotor is influenced by open-to-centre vessels. Increasing the proportion of open-to-centre vessels by shortening stems had no substantial effect in four species. Nor was there more embolism at the segment end versus centre as seen in the Cavitron. The dehydration method yielded an 'r' shaped curve in *Quercus gambelii* that was similar to centrifuged stems with 86% open-to-centre vessels. Both 'r' and 's' (sigmoidal) curves from *Cercocarpus intricatus* were consistent with each other, differing only in whether native embolism had been removed. An 'r' shaped centrifuge curve in *Olea europaea* was indistinguishable from the loss of conductivity caused by forcing air directly across vessel end-walls. We conclude that centrifuge curves on long-vesselled material are not always prone to the open vessel artefact when the original rotor design is used, and 'r' shaped curves are not necessarily artefacts. Nevertheless, confirming curves with native embolism and dehydration data is recommended.

Key-words: Cavitron; plant hydraulics; plant water transport; xylem cavitation and embolism; xylem transport.

INTRODUCTION

Vulnerability curves reveal the decrease in xylem hydraulic conductivity with increasingly negative xylem pressure. The conductivity declines because more of the xylem conduits cavitate and become gas filled, or embolized. A documented cause of the cavitation is air bubbles pulled into the water-filled conduits from adjacent air spaces through the conduit wall (the air-seeding mechanism; Zimmermann 1983). The most likely air entry points are inter-vessel pits between water- and air-filled (already embolized) conduits. Numerous methods have been developed for measuring vulnerability curves, and normally there is good agreement between them (Sperry & Tyree 1990; Cochard, Cruiziat & Tyree 1992; Jarbeau, Ewers & Davis 1995; Cochard *et al*. 2005). However, in angiosperm material with large xylem vessels (tropical trees, temperate ring-porous trees, woody vines and roots), some methods are reported to yield anomalous vulnerability curves which show large losses of xylem conductivity at modest negative pressures (Choat *et al*. 2010; Cochard *et al*. 2010). These so-called 'r' shaped curves (e.g. see Fig. 2, *Quercus gambelii*) have been attributed to what has been termed an 'open vessel' artefact. We investigate the importance of this artefact for the original centrifugal vulnerability curve method (Pockman, Sperry & O'Leary 1995; Alder *et al*. 1997).

The most explicit documentation of an open vessel artefact comes from the Cavitron device (Fig. 1) which was designed for the 'flow-centrifugation' method developed by Cochard and colleagues (Cochard 2002; Cochard *et al*. 2005). As with the original centrifuge method, stem segments are held in a custom-designed rotor so that the ends of the segment are immersed in water-filled reservoirs during spinning (Fig. 1). The spinning induces a parabolic drop in water pressure from atmospheric pressure at the reservoir water surface to a maximally negative pressure at the stem centre. In the flow-centrifugation method, flow is induced through the stem so that hydraulic conductivity can be measured while the stem is spinning. In the original method, there is no flow induced during spinning, and the conductivity is measured outside of the centrifuge between spinning treatments (Alder *et al*. 1997).

There is little doubt that when the Cavitron device is used, long vessels that extend from the stem end to near the segment centre and beyond (referred to here as 'open-to-centre vessels') become artificially vulnerable to cavitation, creating 'r' shaped curves. The strongest evidence comes from stem length experiments, where as stems become

Correspondence: J. S. Sperry. E-mail: j.sperry@biology.utah.edu

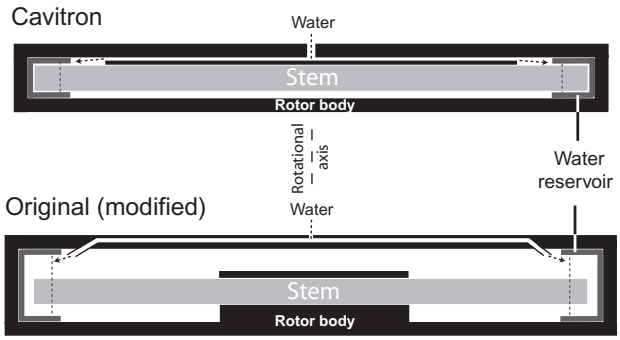

Figure 1. Centrifuge rotor design in the Cavitron apparatus (upper), and in the original rotor (lower; Li *et al*. 2008). Side view only, showing position of stem and water reservoirs within the rotor body. Inlets for water delivery are shown (dashed arrows) for inducing flow and measuring conductivity during spinning. Holes in the reservoirs (not shown) allow the water level to be higher in the upstream than the downstream reservoir, thus inducing flow. Normally with the original design no flow is present and conductivity is measured after spinning; water delivery is a later modification to the original design. Rotors and protocols are described in Alder *et al*. (1997), Cochard (2002), Cochard *et al*. (2005) and Li *et al*. (2008).

shorter, they become more vulnerable to cavitation when spun in the Cavitron (Cochard *et al*. 2010). For example, 27 cm long *Prunus persica* stems lost 50% of their conductivity (PLC_{50}) at about –3.5 MPa, whereas 17 cm long stems had a PLC_{50} of –1 MPa. Maximum embolism was found at the upstream end of the spinning stem rather than in the centre where the most negative xylem pressures were induced. The effect of stem length was much diminished or eliminated when open-to-centre vessels were filled with air prior to spinning. These results are consistent with nucleating agents in the stem water reservoirs (probably microbubbles) being swept into the upstream end of the stem and penetrating far enough into the negative pressure region to nucleate cavitation and the emptying of the entire vessel. In material with small conduits, these nucleators are filtered out by end-walls before reaching the zone of significant negative pressure (Cochard *et al*. 2010).

Does the open vessel artefact also plague the original centrifuge method? There are two features of the original method that could make a difference (Pockman *et al*. 1995; Alder *et al*. 1997). Firstly, there is no flow induced during centrifugation, and so there would be less chance of contaminants being swept into the negative pressure zone. Secondly, the original method typically uses a different rotor design (Fig. 1, Original) where the ends of the segment may be less likely to be exposed to microbubbles or other contaminants (see Discussion). The original method and rotor design have been used extensively, and so it is important to test it just as rigorously for possible artefacts. Previous studies are equivocal in that some show support for the method (Li *et al*. 2008; Taneda & Sperry 2008) while others indicate that an open vessel artefact may exist (Choat *et al*. 2010).

We tested the original method with the same experiments used to expose the open vessel artefact in the Cavitron (Cochard *et al*. 2010). The effect of stem length on vulnerability curves was determined in four species of widely varying vessel length, including the same population of *P. persica* tested in the Cavitron. We also determined whether embolism was maximized at the segment end, as seen in the Cavitron, or in the segment centre, as predicted in the absence of an open vessel artefact. In one species, *Q. gambelii*, we compare centrifugal curves against the original 'dehydration' method (Sperry 1986) compiled from native conductivities and drying branches. Although tedious, dehydration curves are still regarded as something of a 'gold standard' because the embolism is induced naturally by transpiration. We also determined the effect of flushing stems (to remove native embolism) on vulnerability curve shape.

In a final test, we compared an 'r' shaped centrifuge vulnerability curve against a curve obtained by 'single-ended air injection' of the same material. In this injection method, embolism is induced in hydrated xylem by forcing air across inter-vessel pits. Negative pressure is avoided, as are ambiguities associated with the related pressure-sleeve injection method (Choat *et al*. 2010; Ennajeh *et al*. 2011). Single-ended injection has been shown to match embolism caused by passive dehydration of cut branches, providing some of the strongest support for the inter-vessel air-seeding mechanism for cavitation (Sperry & Tyree 1990; Jarbeau *et al*. 1995).

MATERIALS AND METHODS

Plant material

Q. gambelii, *Acer negundo*, *Sorbus scopulina* and *Cercocarpus intricatus* were collected from natural populations in Utah. The first three species were obtained from the Wasatch Mountains near Salt Lake City (ca. 40°46″N 111°48″W, elev. 1630–2030 m), and the last were from southern Utah near Natural Bridges National Monument (ca. 37°51″N 110°12″W, elev. 1700 m). *P. persica* and *Olea europaea* were express mailed from Europe. *P. persica* was provided by Herve Cochard from the same population tested in Cochard *et al*. (2010). *O. europaea* was provided by Antonio Diaz-Espejo from an experimental orchard near Seville, Spain. For all species, branches were cut from several plants in a single population and wrapped tightly in plastic bags before transport to the laboratory at the University of Utah in Salt Lake City. *Sorbus* and *Prunus* were collected in early spring prior to bud break; other species were collected during the growing season.

Centrifuge vulnerability curves

Stem segments were cut from the collected branches under water, trimmed with a fresh razor blade and in most cases, flushed with filtered (0.2 μm) 20 mM KCl solution made up in purified water (distilled water passed through deionizing

Table 1. Vessel length statistics

Species	Median (m)	Mean (m)	Log mean (m)	x = fraction open at length L (m)
Quercus gambelii	0.105	0.174	0.085	$x = e^{-(15.1\ L)^{\wedge}0.66}$
Acer negundo	0.021	0.031	0.019	$x = e^{-(82.8\ L)^{\wedge}0.71}$
Sorbus scopulina	0.039	0.047	0.035	$x = e^{-(43.1\ L)^{\wedge}0.92}$

Data from Wheeler *et al*. (2005) (*Sorbus scopulina*), Sperry, Hacke & Wheeler (2005) (*Acer negundo*) and Hacke *et al*. (2006) (*Quercus gambelii*). Length distributions are short skewed (median is shorter than mean), so the mean of log-transformed data is also given. The Weibull function for x = the fraction of open vessels at length L (Eqn 1, L in m) is given for each species. This value was used to calculate the % of vessels at the centre of the stem that extended to *either* end of the segment ('open-to-centre' vessels, Eqn 2).

and organic removal cartridges; Barnstead E-pure, Thermo Scientific, Waltham, MA, USA). Stems were flushed for at least 30 min at ca. 75 kPa to remove native embolism or any additional embolism induced during harvesting or transport. In *Sorbus* and *Cercocarpus*, some stems were not flushed for comparison. Segments were typically 7–10 mm in diameter and 1–3 years of age (1–2 years in *Q. gambelii*).

Stems were attached to a tubing apparatus filled with the same 20 mM KCl solution, and flow rate induced by a hydraulic head was measured gravimetrically. The head was small enough not to displace air from any vessel that might be continuous through the segment (head < 2T/r, T = surface tension, r = radius of vessel). Stems were either submersed in water or covered with a damp cloth to prevent water loss from the surface. To make a measurement, the head was set to zero, and non-pressurized flow was measured. Typically, this was a small 'negative' flow, meaning the stem was absorbing water. The head was then increased, and pressurized flow was measured. The zero head reading was then repeated. If it was similar to the initial zero head reading, the two zero head flows were averaged and subtracted from the pressurized flow to obtain the net flow rate induced by the pressure difference. If the zero head flow had shifted after the pressurized measurement, the sequence was repeated until a stable zero flow was obtained. The hydraulic conductivity was calculated from the net flow divided by the pressure gradient (head/stem length). Accounting for zero-pressure flow, or otherwise measuring the slope of the flow by pressure relationship, is important for accurate measurements. Otherwise, significant zero-pressure flow will cause errors, and what might seem to be changes in conductivity can actually result from changes in the non-pressurized flow. The error increases in material with inherently low conductivity where pressurized flow rates are small. Occasionally, this calculation yields slightly negative conductivities when total cavitation renders negligible pressurized flow.

After the conductivity measurement, stems were secured in a custom-built rotor (plans available from senior author) that allows them to be spun on a centrifuge (Sorvall RC-5C; Thermo Fisher Scientific) with their ends submersed in water in Plexiglas reservoirs (see Fig. 1; Alder *et al*. 1997). Rotors were available in two sizes, one to fit 27 cm stems and one to fit 14 cm ones. The smaller rotor was also provided with special reservoirs allowing 10 cm stems to be used. After spinning to induce the desired negative pressure at the stem centre for 15 min, stems were removed and the conductivity was measured as before. The process was repeated at progressively greater spinning speeds (more negative pressure) until conductivity approached zero and most of the xylem had been cavitated. For each species and stem length (see below), $n = 6$ stem segments were used. Composite vulnerability curves were plotted either as percentage loss of conductivity (PLC) relative to the initial value, or as hydraulic conductivity divided by stem cross-sectional area (stem-specific conductivity).

Stem length tests

In four species (*Q. gambelii*, *A. negundo*, *S. scopulina*, *P. persica*), we conducted vulnerability curves at stem lengths of 27 and 14 cm. In *Sorbus*, we also measured 10 cm stems. Stems were from the same population, and collected at the same time, and treated identically in every respect except for length. Vessel length distributions for *Quercus*, *Sorbus* and *Acer* were obtained from previously published data on populations from the same Wasatch Mountain locality and for similar stem ages as the centrifuged stems (Table 1). The method for obtaining these distributions is described in Christman, Sperry & Adler (2009). This method yields a Weibull function for the fraction (x) of vessels exposed at one end of a branch segment that extend to any distance L from that end:

$$x = e^{-[(a\,L)^b]} \quad (1)$$

where a and b are the Weibull function parameters. Using this equation, the percentage of vessels extending from the middle to *either* end ($\%L_m$) can be derived:

$$\%\,L_m = 100(2x - x^2) \quad (2)$$

where x is evaluated for L_m, the distance from the middle of the stem to the end. The $\%\ L_m$ in this case is the percentage of 'open-to-centre' vessels in the segment.

Embolism position tests

To determine where embolism was induced in stem segments, *S. scopulina* stem segments 27 cm in length were

spun to induce ca. 80% PLC relative to their initial conductivity, and then cut into three 4 cm segments: two at either end, and one in the centre. The conductivity of each 4 cm segment was measured, then each segment was flushed to reverse embolism, and the PLC of the segment was calculated relative to the flushed value. In the absence of an open-vessel artefact, PLC should always be greatest in the central segment exposed to the most negative xylem pressure. To test whether flushing the stems before centrifugation had any effect on embolism, we repeated the experiment on $n = 4$ branches that were flushed initially, versus $n = 4$ branches that were not flushed initially.

Comparison of dehydration versus centrifuge curves

In *Q. gambelii*, a dehydration vulnerability curve was assembled from both native conductivity and stem xylem pressure measurements and dry-downs of detached saplings beyond their typical xylem pressure range. These measurements were done on a population of 4 to 7-year-old saplings as part of a related study of cavitation by air-seeding in the species (Christman, Sperry & Smith in press). Stem segments used in this comparison were 3–5 years old and thicker (>10 mm diameter) than the 1 to 2-year-old segments measured for the stem-length tests and vessel-length measurements described previously for *Q. gambelii*. Therefore, a separate vessel length distribution was measured for this population and older segment size as detailed in the related study (Christman *et al*., submitted for publication).

To measure native conductivity, a portion of the main axis longer than 27.5 cm was cut from saplings in the field under water. A split funnel was sealed around the sapling and filled with distilled water. The sapling was cut under the water and rapidly transferred (<1 s) into a tray of water where the other end was cut under water. The excised stem segments were kept under water in plastic bags for transport to the laboratory. In the lab, stems were trimmed to 27.5 cm under water and the hydraulic conductivity was measured. The corresponding stem xylem pressure was estimated from pressure chamber (PMS Instruments, Santa Barbara, CA, USA) measurements on bagged leafy shoots adjacent to the stem segment. Three leaves or short-shoots were sampled per tree. Leaves were covered with aluminium foil for at least 20 min prior to collection to promote equilibration of leaf with stem water potentials. Leaves were excised prior to removing the stems for conductivity measurements. Leaves were sampled below the conductivity segments to avoid the possibility of creating emboli in stems as leaves were cut for pressure measurements. Collections were made at pre-dawn and midday to cover the physiological range of native stem xylem pressures.

To obtain negative pressures below the native range, saplings were covered in humidified plastic bags and cut at the base in air before rapidly being transported to the laboratory. Prior to bagging and cutting, three leaves per branch were covered in aluminium foil for subsequent measurement of stem pressures. In the laboratory, branches were dried to a range of xylem pressures. At this point, the xylem pressure was measured on the three bagged leaves, and a 27.5 cm long conductivity segment cut from the branch underwater. The conductivity segment was located far enough from the cut end of the branch (>1 m) to prevent detectable embolism during the initial cut. A Weibull function was fit to the dehydration data, yielding an estimate of the mean and median (P50) cavitation pressure. To propagate uncertainty in the curve fit to these curve-derived statistics, we bootstrapped (randomly sampled with replacement) the dehydration data set to obtain 1000 mean and median pressures. The 2.5th and 97.5th percentiles yielded 95% confidence intervals for these statistics.

A centrifuge vulnerability curve was done for $n = 5$ stem segments of the same size (27.5 cm) and age (3–5 years) for the same population and time period. Native conductivity was obtained in the same manner described for the dehydration curve. Stems were not flushed, so the vulnerability curve corresponded to the xylem functional at the time of collection (for direct comparison to the dehydration curve). Stems were collected at pre-dawn to insure that they were maximally hydrated. The conductivity was normalized by the cross-sectional area of the stem. A Weibull curve fit to each stem yielded five estimates of mean and median cavitation pressure, from which the grand mean, standard error and 95% confidence intervals were determined.

Comparison of vulnerability curves on flushed versus non-flushed stems

In *C. intricatus*, vulnerability curves on 27 cm segments were completed for $n = 6$ stems from the same population that were not flushed prior to the experiment versus flushed. Vulnerability curves were plotted as PLC relative to the initial value and as stem-specific conductivity.

Comparison of centrifuged vulnerability curve versus single-ended air injection vulnerability curve

In *O. europaea*, a centrifuge curve was done on $n = 6$ vacuum-infiltrated segments of 27 cm length and 2–3 years old. This was compared with a curve produced using the single-ended air injection method (Sperry & Tyree 1990). Branches cut at the base but otherwise intact (except for leaf removal) were vacuum infiltrated in 20 mM KCl solution for an hour. Infiltrated branches were injected at their base with air using a pressure chamber. Air was injected for 30 min at a single pressure. After injection, a 10 cm long branch segment of similar diameter and age (2–3 years) was cut from the branch under water and its hydraulic conductivity was measured. The proximal end of the segment was located at least 50 cm from the injected end. Four branches were injected at each of four pressures: 0.1, 1, 2 and 3 MPa. The 0.1 MPa injection was chosen to embolize all vessels

exposed at the injection site, but not to penetrate their pitted end-walls. Four control branches were not injected. Conductivity after the 0.1 MPa injection was no different from controls, indicating that the 50 cm buffer distance was sufficient to exclude detectable numbers of open vessels from the conductivity segment. Loss of conductivity at the higher pressures would indicate breaching of inter-vessel pits. Pressures above 3 MPa were not used because of problems with stems shooting out of the pressure chamber.

RESULTS

Stem length tests

We found no major effect of stem length on vulnerability curves for any of the four species tested (Fig. 2) despite a wide range of mean vessel lengths (Table 1, mean lengths 3.1 to 17.4 cm). Stem and vessel lengths determine the percentage of vessels open to the middle of the segment (open-to-centre vessels) where xylem pressures are most negative

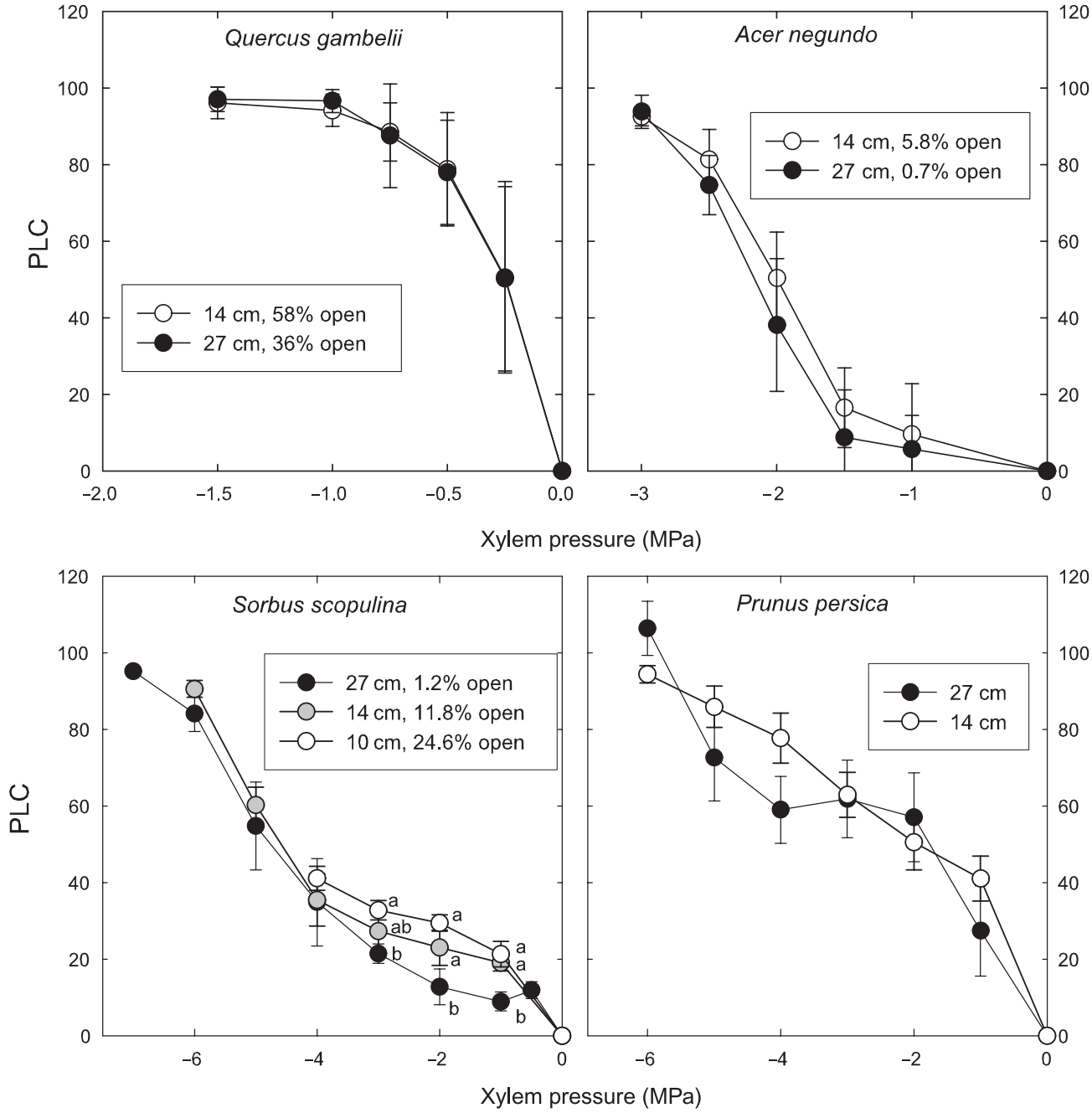

Figure 2. Effect of stem length on centrifuge vulnerability curves in four species. Mean ± SE for $n = 6$ stems. Percentages of vessels extending to the stem centre were calculated from equations in Table 1 for *Quercus gambelii, Acer negundo* and *Sorbus scopulina*. Data points with different letters in the *S. scopulina* figure denote significant differences at $P \leq 0.05$. PLC, percentage loss of conductivity.

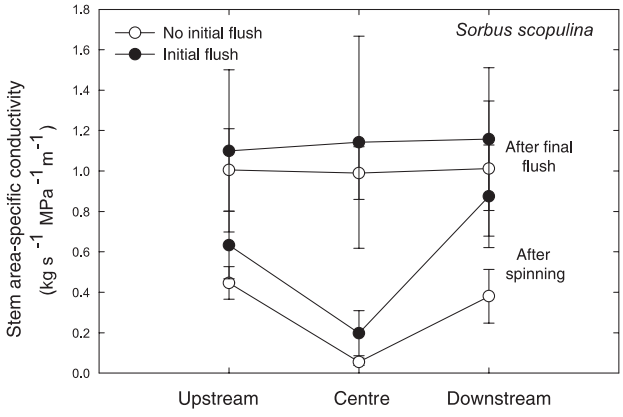

Figure 3. Stem area-specific conductivity in 4 cm *Sorbus scopulina* stem segments cut from a 27 cm branch after spinning to induce >80 PLC. Mean ± SE for $n = 6$ stems. Regardless of whether stems were flushed (open) or not flushed (solid) before spinning, conductivity was lowest in the centre than at either end after spinning (lower curves) relative to the maximum conductivity after a final flush to remove embolism (upper curves). PLC, percentage loss of conductivity.

during centrifugation (Table 1, % open). *Q. gambelii* had a pronounced 'r' shaped curve which was identical for 27 cm segments (estimated 36% open-to-centre vessels) or 14 cm segments (58% open-to-centre vessels). *A. negundo* had an 's' shaped curve that was similarly identical at the two lengths (27 cm, 0.7%; 14 cm, 5.8% open-to-centre). *S. scopulina* had an 's' shaped curve at all three of the tested lengths, with no difference between 14 cm stems (11.8% open-to-centre) or 10 cm ones (24.6% open-to-centre). The 27 cm stems (1.2% open-to-centre) were less embolized at the initial pressures, but the difference was less than 10% and far less than differences reported by Cochard *et al*. (2010). *P. persica* had a more or less linear vulnerability curve that was no different between 14 and 27 cm lengths. The same population tested in the Cavitron showed 17.5 cm segments to be dramatically more vulnerable than 27.5 cm ones (Cochard *et al*. 2010).

Embolism position test

Flushed and non-flushed *S. scopulina* segments of 27 cm length were spun to –4 MPa, which induced substantial embolism. When cut into 4 cm segments, the loss of conductivity was greatest in the central 4 cm where the number of open vessels was least, relative to the 4 cm segments from either end with necessarily more open vessels (Fig. 3). Whether or not stems were flushed initially had no effect on the embolism distribution (Fig. 3, open versus solid symbols).

Comparison of dehydration versus centrifuge curves

Native stem area-specific conductivity in *Q. gambelii* was extremely variable, but showed a decline from pre-dawn (xylem pressure typically ≥0.5 MPa; Fig. 4, open triangles) to midday (pressures to –1.5 MPa, Fig. 4, grey triangles), suggestive of diurnal refilling of embolized vessels. Native conductivities in combination with excised branch dry-downs (Fig. 4, dark grey triangles) yielded a dehydration vulnerability curve. Drying branches below the native pressure range continued the highly variable decline in conductivity. Some branches reached zero conductivity at or above –2 MPa, whereas others maintained limited conductivity to below –4 MPa. Overall, the dehydration curve showed a substantial drop in conductivity between 0 and –1 MPa with a long tail of low conductivity reaching zero below –4 MPa. The shape is equivalent to an 'r' shaped curve if the data were plotted as PLC instead of as area-specific conductivity. Fitting a Weibull function to the entire data set (Fig. 4, solid curve, $r^2 = 0.43$) yielded a mean cavitation pressure of –1.22 MPa (95% CI: –0.77, –1.62) and a median (P50) of –1.08 MPa (–0.44, –1.54).

The centrifuge vulnerability curve done on non-flushed conductivity segments collected at pre-dawn had a similar shape to the dehydration curve (Fig. 4, solid circles), showing a population of vulnerable vessels cavitating between pre-dawn and midday pressures and a long resistant tail. The mean cavitation pressure in the centrifuged stems was –0.63 MPa (95% CI: –0.47, –0.79) and a median (P50) of –0.45 MPa (–0.24, –0.66). These values were 0.59 and 0.63 MPa less negative than the corresponding estimates from the dehydration curve but with overlapping confidence intervals. The centrifuged stem segments had an estimated 86% open-to-centre vessels, a considerably greater fraction in these 3–5 years segments than the 1–2 years segments measured for Table 1 and Fig. 1. Note that aside from its similar 'r' shape, the centrifuge curve in Fig. 4 was not necessarily comparable with the one in Fig. 1

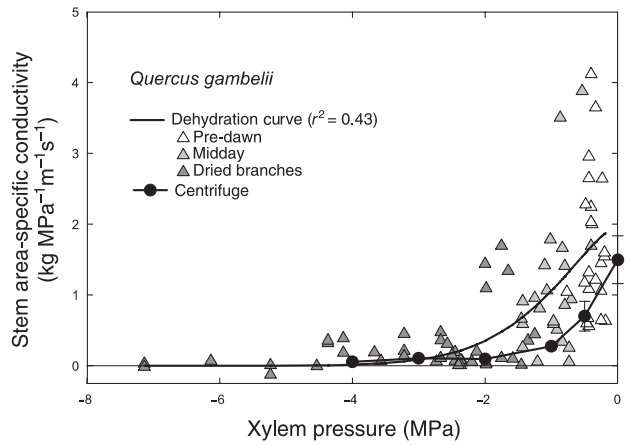

Figure 4. Dehydration vulnerability curve of *Quercus gambelii* (triangles) versus a centrifuge vulnerability curve on the same population. Each dehydration datum is a separate stem: open triangles are stems at pre-dawn, light grey at midday and dark grey were from dehydrated branches. The centrifuge curve was compiled from $n = 5$ stems collected at pre-dawn (means ± SE). Curves are plotted as stem conductivity per cross sectional stem area versus xylem pressure.

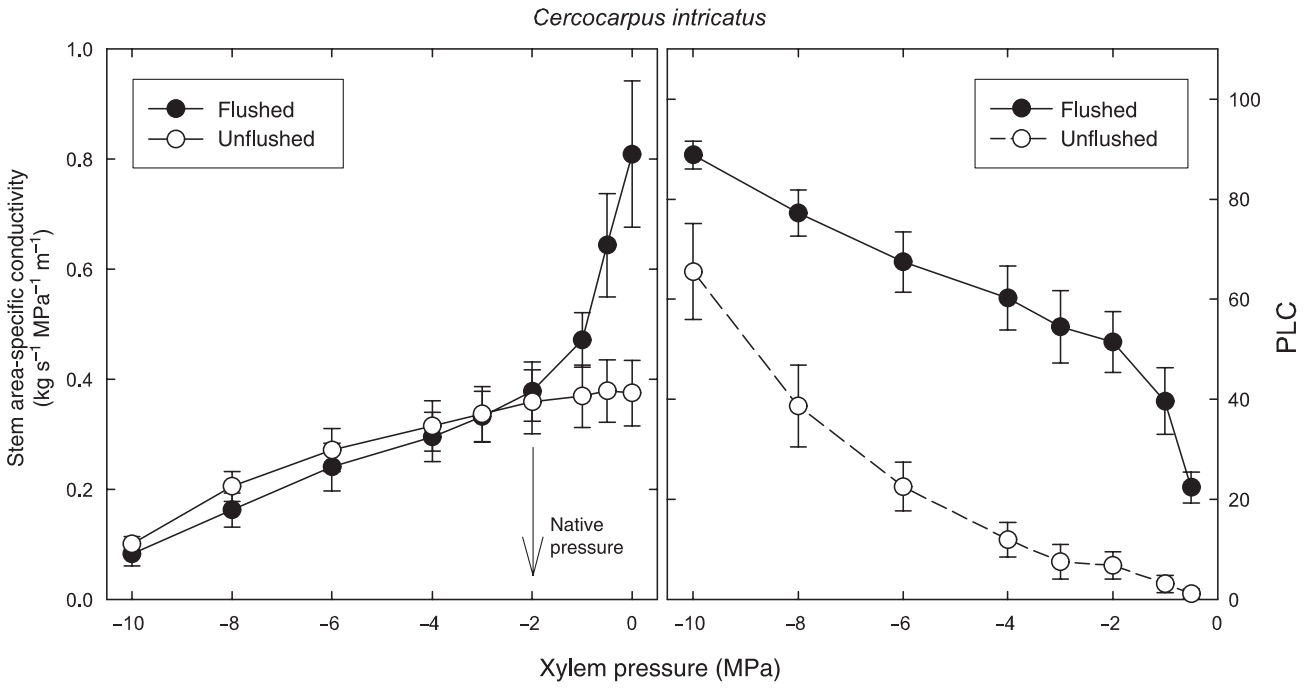

Figure 5. Centrifuge vulnerability curves for flushed (solid) and non-flushed (open) stems of *Cercocarpus intricatus* (mean ± SE, $n = 6$ stems). Curves are plotted as stem area-specific conductivity (left) and as percentage loss of conductivity (right; PLC, relative to initial). Convergence of curves at a predicted native xylem pressure of –2 MPa is evident in the area-specific conductivity curves (left), but not in the PLC curves (right).

because of differences in stem age, collection site, and whether the stems were flushed (Fig. 1) or not (Fig. 2).

Effect of embolism removal on shape of vulnerability curves

Flushed stem segments (14 cm) of *C. intricatus* had greater stem-area specific hydraulic conductivity than non-flushed segments harvested simultaneously from the same individuals as a result of the reversal of native embolism. When vulnerability curves were plotted as conductivity per stem area (stem-specific conductivity), conductivity of the flushed segments fell by 50% to converge on the native conductivity at –2 MPa (Fig. 5, left panel). The –2 MPa value is a typical xylem pressure for a semi-arid shrub of the Colorado Plateau region (Gebauer, Schwinning & Ehleringer 2002). Below –2 MPa, the two curves were identical. This result indicates a native embolism level of about 50 PLC as a result of natural cavitation caused by xylem pressures reaching –2 MPa in the field. The results suggest that both curves are equally accurate measures of xylem vulnerability. The flushed vulnerability curve reflects cavitation in all of the xylem, embolized plus non-embolized. The non-flushed curve reflects only cavitation in the non-embolized xylem.

When these data are plotted as PLC (Fig. 5, right panel), the basic congruence of the two conductivity curves is not evident. The flushed curve, with an 'r' shape, appears to show much more vulnerable xylem than the broad 's' shape of the non-flushed curve. The PLC metric in this example obscured the fact that both curves show identically vulnerable xylem below –2 MPa.

Comparison of 'r' shaped centrifuge curves with single-ended air injection results

Vacuum-infiltrated *O. europea* stem segments of 27 cm in length had centrifugal vulnerability curves with an initially steep loss of conductivity (Fig. 6). Although plotted as stem-specific conductivity, this curve would have the 'r' shape if plotted as PLC (e.g. see also Figs 4 & 5, flushed stems). Segments of similar diameter and age cut from vacuum-infiltrated and air-injected branches yielded an 'r' shaped curve that was indistinguishable from the centrifuged stems (Fig. 6, open symbols). Lack of any drop in conductivity at the initial low pressure injection of 0.1 MPa indicated that the conductivity segments were sufficiently distant from the injection site to be protected by vessel end-walls. The subsequent drop in conductivity at higher air pressures represented air seeding through intact end-walls. This measure of the permeability of the end-walls to air seeding matched the development of embolism in the centrifuged stems.

DISCUSSION

Our results provided little evidence for an open vessel artefact when using the original centrifuge method with the original rotor design (Alder *et al*. 1997; Li *et al*. 2008). No

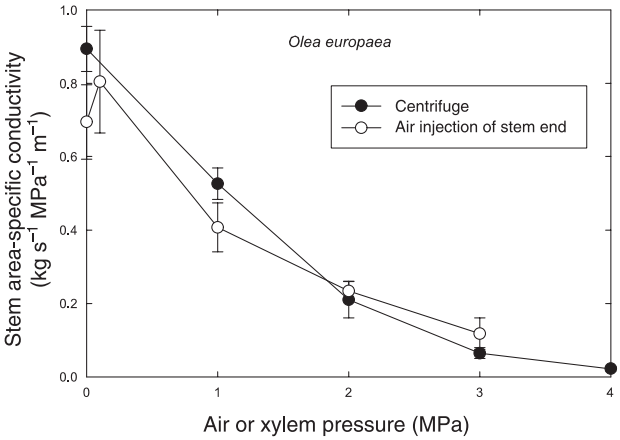

Figure 6. Comparison of centrifuge vulnerability curve (solid) and single-ended air-injection curve (open) in *Olea europaea* (mean ± SE, $n = 6$ stems). Curves are plotted as stem area-specific conductivity.

major effects of stem length were observed in four species of diverse vessel length (Fig. 2; Table 1), not even in the same species and population (*P. persica* from Clermont Ferrand, France) that showed dramatic response to stem shortening in the Cavitron (Cochard *et al*. 2010, their fig. 3). The small effect we did observe could have resulted from embolized central vessels making up proportionally more of the stem length in short versus long stems (Cai & Tyree 2010). Within a centrifuged segment, embolism was lowest at the ends of the stems relative to the centre (Fig. 3) despite the ends containing more open vessels than the centre. Again, this result is directly opposite to Cochard *et al*. (2010, their fig. 5), finding anomalously high embolism at the upstream end of the segments spun in the Cavitron. Finally, the 'gold standard' dehydration method yielded an 'r' shaped curve in *Q. gambelii* that was similar in shape to the centrifuge curve (Fig. 4).

Why the difference in results? There is little doubt that the Cavitron suffers from an open vessel artefact based on the very different results from the same experiments (and same species) we have tested with the older rotor design. A potential reason is that flow is induced during the Cavitron measurement method which could sweep contaminants into the stem. In the original method, there is no induced flow during spinning. However, the presence versus absence of flow cannot be the only explanation for the Cavitron anomaly. Li *et al*. (2008) modified the original rotor so that conductivity could be measured during spinning as in the Cavitron (Fig. 1, addition of water delivery system to original design). With the modified original rotor, they compared vulnerability curves done with the original (no flow) method versus the flow-through method for 10 species of extremely diverse conduit length (two conifer, four diffuse-porous, four ring-porous). If the induction of flow was causing an open vessel artefact, the flow-through curves should have been more vulnerable than the no-flow curves in the longer-vesselled species. However, no major differences were found in any species, suggesting that the presence of flow during centrifugation in the original rotor was not influencing the results.

The anomalous Cavitron results may be a consequence of rotor design. As shown in Fig. 1, in the Cavitron, the stem is wedged tightly in a covered rectangular slot but otherwise not secured. The stem ends are enclosed in tight-fitting plastic reservoirs. During spinning, water is delivered from a central port to the reservoirs (Fig. 1, dashed arrows in upper diagram). Because of the tight fit, any microbubbles created by the turbulence of this delivery would be in close proximity to the stem end and could be swept into the upstream end. In contrast, the design of the original rotor has much larger reservoirs because the stem is held at mid-length (Fig. 1, bottom diagram). Water delivery to the reservoirs during spinning (when flow-through measurements are made) is remote from the stem end (dashed arrows), and bubbles created by turbulence are unlikely to enter the stem and cause problems.

The strongest evidence for the validity of an 'r' shaped curve is the 'r' shaped dehydration curve for *Q. gambelii* (Fig. 4). Pre-dawn conductivities in *Q. gambelii* were greater than midday values, indicating diurnal embolism and refilling cycles in a population of vulnerable vessels. This refilling behaviour is described in more detail in a related study (Christman *et al*. 2011). The dehydration curve also indicated a set of much more resistant vessels that maintained conductivity to below –4 MPa in some stems. Although the mean and median cavitation pressures from the dehydration curve were more negative than from the centrifuge curve, the 95% confidence intervals for these statistics overlapped. If the difference between the curves resulted from a centrifuge artefact, it is a much more subtle one than seen with the Cavitron device, considering that 86% of the vessels in the centrifuged stems were open-to-centre.

Arguing against a major centrifuge artefact are previous data from *Q. gambelii* showing that native embolism matched the centrifuge vulnerability curve, and that spinning stems at their native xylem pressure produced a similar loss of conductivity (relative to a flushed maximum) as seen in the same stems in their native state (Li *et al*. 2008; Taneda & Sperry 2008). There are other possible explanations besides a centrifuge artefact for the apparent offset between the centrifuge and dehydration curves in *Q. gambelii*. The conductivity of the centrifuged stems was relatively low before the stems were even subjected to spinning (Fig. 1, y axis intercept of centrifuge curve), suggesting that the offset could be in part due to the chance sampling of low-conductivity stems. Also, the dehydration data could be shifted to erroneously negative xylem pressures if the bagged leaf xylem pressure was not fully equilibrated with stem pressure. Leaves were bagged at least 20 min prior to measurement, which may not be sufficient to achieve complete equilibration under all circumstances (Pratt & Tobin, personal communication).

As seen in the *C. intricatus* result (Fig. 5), an 'r' shaped curve is only possible if native embolism is reversed beforehand; otherwise, the curve must be 's' shaped if there

is no open vessel artefact. Unless embolism is removed prior to curve generation, the vulnerability of any previously embolized xylem is unknowable, and in this sense curves on flushed or vacuum-infiltrated material are more informative. While some of the embolized xylem may be from past seasons and rendered more vulnerable by 'cavitation fatigue', some of it may be from the current season. When it is desirable to exclude fatigued xylem from the calculation of median or mean cavitation pressures, flushed curves can be converted into non-flushed curves by scaling them to native xylem pressure (Pockman & Sperry 2000; Hacke *et al*. 2006). Perhaps the ideal for making cross-species comparisons would be curves that start at the seasonal maximum pre-dawn xylem pressure and native conductivity, as in the *Q. gambelii* example of Fig. 4.

Although plotting vulnerability curves as PLC and citing mean or median (P50) cavitation pressures has proven utility, in some cases it is more informative to focus on the actual area-specific conductivity. In the *C. intricatus* example, the PLC curves (PLC relative to initial conductivity) obscured the substantial overlap between the flushed 'r' and non-flushed 's' curves that is evident when stem-specific conductivities are plotted (Fig. 5). However, this deficiency would be remedied if non-flushed PLC was calculated relative to a flushed maximum determined after spinning (analogous to many PLC dehydration curves). From a technical standpoint, PLC is more error prone because it can be confounded by any shift in 'maximum' conductivity caused by inadequate embolism reversal or irreversible plugging of embolized vessels (e.g. by tyloses). But most importantly, PLC curves and cavitation pressures can be extremely misleading about the ecophysiological impact of cavitation. The 'r' shaped PLC curve of *Q. gambelii* in Fig. 1 predicts over 80 PLC under normal conditions and has a P50 of ca. –0.25 MPa. This sounds strikingly maladaptive, especially when co-occurring species like *Acer grandidentatum* are operating below 10 PLC with a P50 of ca. –3 MPa (Alder, Sperry & Pockman 1996). But in terms of their actual native stem-specific conductivity, the two species are not that different (Taneda & Sperry 2008). In summary, PLCs and mean or median pressures do not serve to compare conducting capacities when initial conductivities vary widely across species, organ or treatment.

More evidence for the validity of 'r' shaped curves comes from the *O. europaea* results. The 'r' shaped centrifuge curve for this species was exactly matched by embolism caused by single-ended air injection (Fig. 5). Single-ended air injection is an unambiguous test of the vulnerability of vessel end-walls to air penetration because all open vessels at the injected end are exposed to the same air pressure. Single-ended air-injection curves have also been shown to match dehydration curves (Sperry & Tyree 1990; Jarbeau *et al*. 1995), which is expected given the two methods provide the same opportunity for air seeding from the cut branch ends. The fact that over 50% of the olive stem conductivity was lost by injection at pressures above 1 MPa is most easily explained by there being a large fraction of vessels with leaky end-walls that are prone to cavitation by air seeding. The same vulnerable population of vessels is also seen from the centrifuge vulnerability curve as expected if similar opportunities for air seeding exist. Whether these leaky end-walls were the result of cavitation fatigue (weakening from previous air seeding; Hacke *et al*. 2001) or not is unknown, although stem segments were 2–3 years old and could have been partially fatigued.

In puzzling contrast to our results, Choat *et al*. (2010) found that 'r' shaped centrifuge curves (using the original rotor design) grossly overestimated native PLC in *Vitis vinifera*. Dehydration curves were 's' shaped and consistent with low native embolism. These unexplained disparate results between species indicate that great care must be exercised in evaluating validity of vulnerability curves in large-vesselled material even if the original rotor design is used. An 'r' shaped curve is suspicious, and the most practical check is to compare the curve with native conductivity and embolism measurements (i.e. a partial or complete dehydration curve). If the native stem-specific conductivity is greater than predicted from the vulnerability curve (i.e. less embolism than predicted), the curve is likely in error unless refilling occurred prior to the native conductivity measurement or the estimate of stem xylem pressure was too negative.

We conclude that centrifuge vulnerability curves on large-vesselled material are not in each and every case subject to an open vessel artefact when the original rotor design is used. In our material, curves were relatively insensitive to the number of open-to-centre vessels, there was no evidence of anomalous embolism at segment ends, and 'r' shaped centrifuge curves were consistent with 'r' shaped curves from branch dehydration and single-ended air injection. Nevertheless, there is a possibility that centrifugation reduces conductivity relative to the native state (Fig. 4), and there are unexplained discrepancies in other work (Choat *et al*. 2010). Thus, curves on large-vesselled material should be checked against native conductivity measurements or dehydration curves.

ACKNOWLEDGMENTS

We thank Herve Cochard, of INRA (Clermont-Ferrand, France), for discussions, comments on manuscript drafts and mailing of *Prunus persica* material from France. Antonio Diaz-Espejo of IRNASE-CSIC (Seville, Spain) sent *Olea europaea* material. Jim Wheeler did the *Cercocarpus intricatus* vulnerability curves as an undergraduate at the University of Utah. Taylor J. Workman assisted with *Quercus gambelii* vulnerability curves and was supported by the University of Utah Undergraduate Summer Research Program. J.S.S. acknowledges financial support from NSF-IBN-0743148. H.T. was supported by the Japan Society for Promotion of Science to work at the University of Utah. J.M.T-R. was supported by Spanish Ministry of Science and Innovation to visit the University of Utah. Anna Jacobsen and Brandon Pratt (University of

California, Bakersfield) and Uwe Hacke (University of Alberta, Edmonton Canada) provided valuable comments on manuscript drafts.

REFERENCES

Alder N.N., Sperry J.S. & Pockman W.T. (1996) Root and stem xylem cavitation, stomatal conductance, and leaf turgor in *Acer grandidentatum* across a soil moisture gradient. *Oecologia* **105,** 293–301.

Alder N.N., Pockman W.T., Sperry J.S. & Nuismer S. (1997) Use of centrifugal force in the study of xylem cavitation. *Journal of Experimental Botany* **48,** 665–674.

Cai J. & Tyree M.T. (2010) The impact of vessel size on vulnerability curves: data and models for within-species variability in saplings of aspen, *Populus tremuloides* Michx. *Plant, Cell & Environment* **33,** 1059–1069.

Choat B., Drayton W.M., Brodersen C., Matthews M.A., Schackel K.A., Wada H. & McElrone A.J. (2010) Measurement of vulnerability to water stress-induced cavitation in grapevine: a comparison of four techniques applied to a long-vesseled species. *Plant, Cell & Environment* **33,** 1502–1512.

Christman M.A., Sperry J.S. & Adler F.R. (2009) Testing the rare pit hypothesis in three species of *Acer*. *New Phytologist* **182,** 664–674.

Christman M.A., Sperry J.S. & Smith D.D. (2011) Rare pits, large vessels, and extreme vulnerability to cavitation in a ring-porous tree species. *New Phytologist* (in press).

Cochard H. (2002) A technique for measuring xylem hydraulic conductance under high negative pressures. *Plant, Cell & Environment* **25,** 815–819.

Cochard H., Cruziat P. & Tyree M.T. (1992) Use of positive pressures to establish vulnerability curves - further support for the air-seeding hypothesis and implications for pressure volume analysis. *Plant Physiology* **100,** 205–209.

Cochard H., Gaelle D., Bodet C., Tharwat I., Poirier M. & Ameglio T. (2005) Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. *Physiologia Plantarum* **124,** 410–418.

Cochard H., Herbette S., Barigah T. & Vilagrosa A. (2010) Does sample length influence the shape of vulnerability to cavitation curves? A test with the Cavitron spinning technique. *Plant, Cell & Environment* **33,** 1543–1552.

Ennajeh M., Simoes F., Khemira H. & Cochard H. (2011) How reliable is the double-ended pressure sleeve technique for assessing xylem vulnerability to cavitation in woody angiosperms? *Physiologia Plantarum* **142,** 205–210.

Gebauer R., Schwinning S. & Ehleringer J.R. (2002) Interspecific competition and resource pulse utilization in a cold desert community. *Ecology* **83,** 2602–2616.

Hacke U.G., Stiller V., Sperry J.S., Pittermann J. & McCulloh K.A. (2001) Cavitation fatigue: embolism and refilling cycles can weaken cavitation resistance of xylem. *Plant Physiology* **125,** 779–786.

Hacke U.G., Sperry J.S., Wheeler J.K. & Castro L. (2006) Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiology* **26,** 689–701.

Jarbeau J.A., Ewers F.W. & Davis S.D. (1995) The mechanism of water-stress-induced embolism in two species of chaparral shrubs. *Plant, Cell & Environment* **18,** 189–196.

Li Y., Sperry J.S., Bush S.E. & Hacke U.G. (2008) Evaluation of centrifugal methods for measuring xylem cavitation in conifers, diffuse- and ring-porous angiosperms. *New Phytologist* **177,** 558–568.

Pockman W.T. & Sperry J.S. (2000) Vulnerability to cavitation and the distribution of Sonoran Desert vegetation. *American Journal of Botany* **87,** 1287–1299.

Pockman W.T., Sperry J.S. & O'Leary J.W. (1995) Sustained and significant negative water pressure in xylem. *Nature* **378,** 715–716.

Sperry J.S. (1986) Relationship of xylem embolism to xylem pressure potential, stomatal closure, and shoot morphology in the palm *Rhapis excelsa*. *Plant Physiology* **80,** 110–116.

Sperry J.S. & Tyree M.T. (1990) Water-stress-induced xylem embolism in three species of conifers. *Plant, Cell & Environment* **13,** 427–436.

Sperry J.S., Hacke U.G. & Wheeler J.K. (2005) Comparative analysis of end-wall resistance in xylem conduits. *Plant, Cell & Environment* **28,** 456–465.

Taneda H. & Sperry J.S. (2008) A case-study of water transport in co-occurring ring- versus diffuse-porous treses: contrasts in water-status, conducting capacity, cavitation and vessel refilling. *Tree Physiology* **28,** 1641–1652.

Wheeler J.K., Sperry J.S., Hacke U.G. & Hoang N. (2005) Inter-vessel pitting and cavitation in woody Rosaceae and other vesselled plants: a basis for a safety vs. efficiency trade-off in xylem transport. *Plant, Cell & Environment* **28,** 800–812.

Zimmermann M.H. (1983) *Xylem Structure and the Ascent of Sap*. Springer, Berlin, Heidelberg, New York.

Received 23 June 2011; received in revised form 22 September 2011; accepted for publication 25 September 2011